

Exhibit A: Evidence that conjugation through the N-terminus of superantigens prevents MHC class II binding.

Peptides coupled to Zn mediated superantigens such as SPE-C prevent binding to MHC class II

SPE-C containing a trace of radioiodinated SPE-C was coupled to pigeon cytochrome C peptide (PCC) through an N-terminal cysteine introduced at position 1 of SPE-C (SPE-C D1C). A mix of ^{125}I SPE-C D1C and ^{125}I PCC:SPE-C D1C was incubated with human MHC class II bearing cells. MHC class II was immunoprecipitated from detergent lysed cells and run on SDS PAGE and the subjected to autoradiography.

Figure 1 show that the detergent lysate precipitated with either the anti-DR1 mAb L243 (lane 1 and 2) or anti-SPE-C antiserum (lane 3 and 4) precipitated only one band which ran coincident with the uncoupled SPE-C D1C mutant. There was no evidence of the higher molecular weight PCC:SPE-C D1C.

The PCC:SPE-C and SPE-C D1C were clearly distinguished PAGE (lane 7). The ratio of PCC:SPE-C to SPE-C was 50:50 but only the lower MW band bound to MHC class II.

This was evidence that the peptide or any other compound such as a GST fusion coupled to SPE-C at the N-terminus blocked the MHC class II binding site because the two are adjacent to each other.

From this we can extrapolate that superantigens that rely on the zinc site to bind MHC class II such as SPE-C and SMEZ-2 loose their capacity to bind MHC class II if a peptide or protein is attached at the N-terminus. This is because the N-terminus is in close proximity to the Zn atom that is central to MHC class II binding (Figure 2).

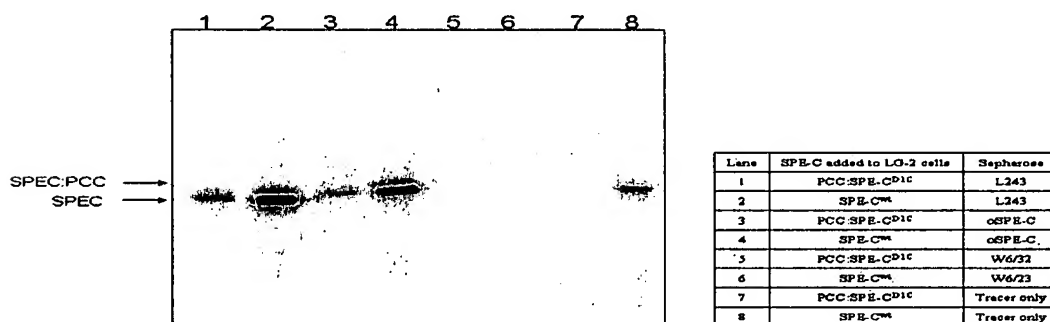


Figure 1 PCC:SPE-C D1C does not bind MHC class II

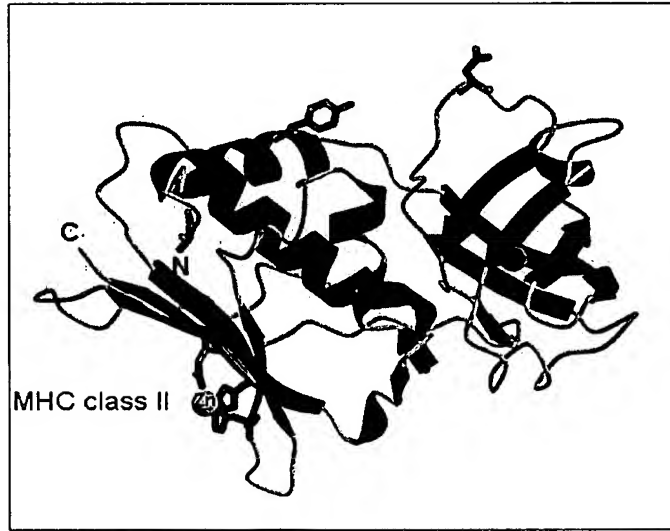


Figure 2: Location of N-terminus to Zn site in SPE-C

A fusion protein of thioredoxin-SMEZ-2 M1 does not bind MHC class II.

SMEZ-2 M1 is produced from *E. coli* as an N-terminal fusion protein with the 10 kD thioredoxin. MHC class II bearing LG-2 cells were incubated with 1 ng 125 I SMEZ-2. And 1- 10,000 ng of either unlabelled thioredoxin-SMEZ-2 M1 or SMEZ-2 M1. Cells were lysed and MHC class II was precipitated with the anti-DR1 mAb L243. Radiolabelled SMEZ-2 was determined in a gamma counter.

The results showed that SMEZ-2 M1 competed for binding but the TX-SMEZ-2 displayed no competitive inhibition. Thus N-terminal fusion proteins to zinc mediated superantigens lose their ability to bind to MHC class II.

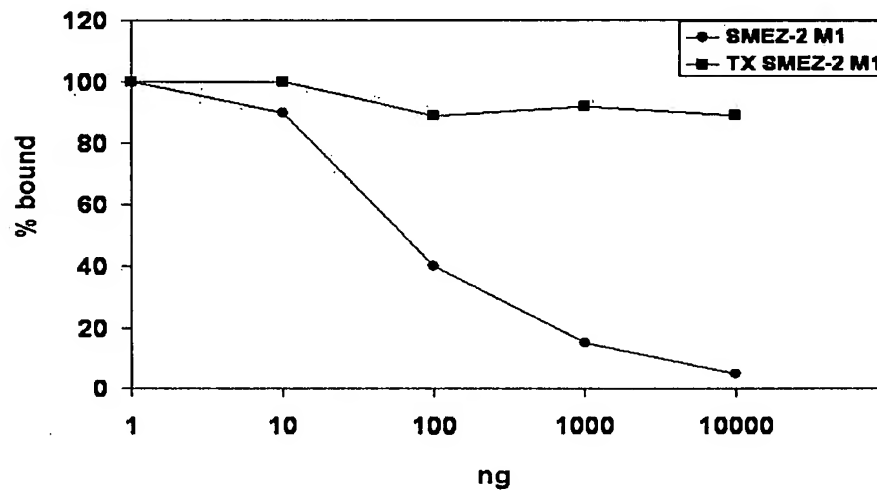


Figure 3: Competitive inhibition by SMEZ-2 M1 but not thioredoxin-SMEZ 2 M1

Antibody response to HIV p7 peptide.

The HIV p7 nucleocapsid peptide was conjugated to SMEZ-2 M1 and injected into test mice. Groups of mice were independently primed and boosted with the p7 alone, peptides in addition to, and peptides coupled with, SMEZ-2 M1. All antigens were in Incomplete Freund's Adjuvant (IFA). Interim analysis of the serum has been done to follow the quantitative antibody response to the peptides. This will be followed up by full term antibody response analysis and additional qualitative antibody isotype analysis.

The antibody response to the p7 peptide showed that no response was found at all, until p7 was coupled to SMEZ-2 M1 and then a good antibody response was achieved. At 10 weeks, all mice had suitable titres of anti-p7 antibodies well above those mice who received p7 peptide alone.

Anti-HIV p7 total Ig in mice

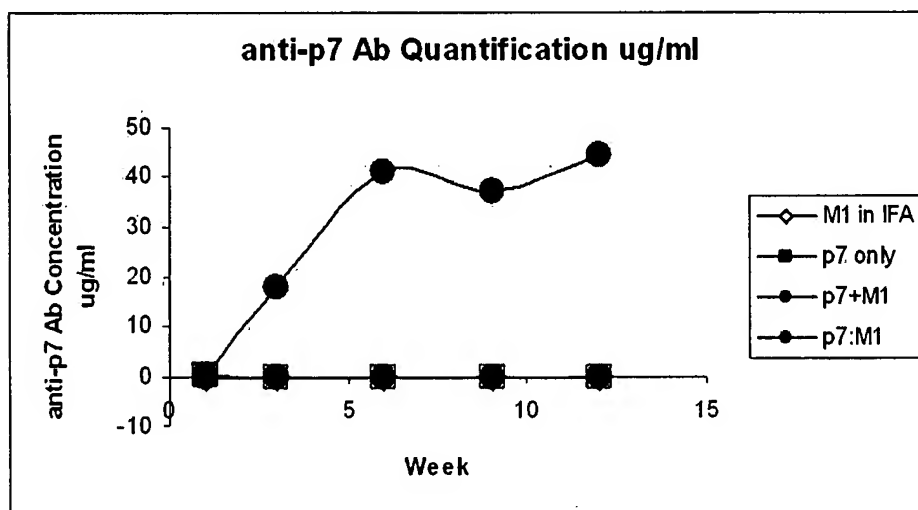


Figure 4: Antibody response to HIV p7 peptide in mice